Out of the

Division of Infectious Diseases and Tropical Medicine, Medical Centre of the University of Munich (LMU)

Effectiveness of prevention of mother-to child transmission (PMTCT) procedures in pregnant HIV infected women and their exposed infants at seven health centers in Addis Ababa, Ethiopia

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Abstract

Background:

The purpose of this research is to assess PMTCT intervention uptake by HIV positive pregnant mothers and to assess the degree of linkage to HIV exposed infants to care and treatment services in selected. We conducted a prospective observational study in HIV positive pregnant mothers and their new-borns attending ANC and PMTCT health services at seven health centers in Addis Ababa, Ethiopia.

Methods:

Outcome evaluations included retention to PMCTC procedures, timing of ART initiation, self-reported ART adherence using a 5-item recall questionnaire, risk factors associated with poor treatment adherence, up-take of infant nevirapine prophylaxis, HIV early infant diagnosis (EID) procedures and mother-to-child transmission rates.

Results:

Of 494 women enrolled into the study 4.9% did not complete PMTCT procedures due to active refusal or loss to follow-up. First HIV diagnosis was done in 223 (45.1%) and ART initiated in 321 (65.0%) women during pregnancy. The median time of ART initiation after HIV diagnosis was 1.3 weeks (IQR 0-4.3) and 17.4 weeks (IQR 11.7-23.9) before delivery. The majority received triple ART, except 37 (7.6%) women starting zidovudine monotherapy following Option A procedures. Poor self-reported treatment adherence was higher post-partum as compared to prior delivery (12.5% versus 7.0%, p=0.002), and significantly associated with divorced/separated marital status (RR 2.2, 95% CI 1.3-3.8), low family income (RR 2.1, 95% CI 1.1-4.1), low CD4 count (RR 1.7, 95% CI 1.0-3.0), and ART initiation during delivery (RR 2.5, 95% CI 1.1-5.6). Of 435 infants born alive 98.6% initiated nevirapine prophylaxis. The mother-to-child HIV transmission rate was 0.7%, but EID results were received only in 4.8% within 2 months, and cumulatively 46.6% within 3 months after birth.

Conclusion: High retention to PMTCT services, triple maternal ART and high uptake of infant nevirapine prophylaxis was associated with a low mother-to-child HIV transmission rate. Declining post-partum antiretroviral treatment adherence and challenges of EID linkage procedures require focused interventions.

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Abbreviation

ART	Antiretroviral Treatment
3TC	Lamivudine
AA	Addis Ababa
ANC	Antenatal Care
APGAR	Appearance, Pulse, Grimace, Activity, Respiration
ARV	Antiretrovirals
AZT	Zidovudine
CD4	Cluster of Differentiation 4 for the T-helper cell
CDC	Center for Disease Control
DBS	Dry Blood Spots
DHS	Demography Health Survey
DRC	Democratic Republic of Congo
EFV	Efavirenz
EID	Early Infant Diagnosis
eMTCT	Elimination of Mother to Child Transmission
EPI	Extended Program for Immunization
EPRDF	Ethiopian People's Revolutionary Democratic Front
FMOH	Federal Ministry of Health
GAFTAM	Global Fund to Fight AIDS Tuberculosis and Malaria
HAART	Highly Active Antiretroviral Treatment
HEP	Health Extension Program
HIV	Human Immunodeficiency Syndrome
HIV RNA	HIV Virus RNA – used to measure Viral Load
HSDP	Health Sector Development Programs
M&E	Monitoring and Evaluation
MDG	Millennium Development Goals
MMWR	Morbidity and Mortality Weekly Report
MTCT	Mother to child transmission
NGO	Non- governmental Organization

NVP	Nevirapine
OI	Opportunistic Infections
PACTG	Pediatric AIDS Clinical Trial Group
PCR	Polymerase chain reaction
PEPFAR	President's Plan for Emergency AIDS Relief
PHCU	Primary Health Care Unit
PLWHA	People Living With HIV and AIDS
PMTCT	Prevention of Mother to Child transmission
PrEP	Pre-exposure prophylaxis
sdNVP	Single dose Nevirapine
SNNP	Southern Nation Nationalities and People Region
SSA	Sub –Sharan Africa
STI	Sexually Transmitted Infection
TB	Tuberculosis
TDF	Tenofovir
UNAIDS	Joint United Nations Programme on HIV/AIDS
VCT	Voluntary Counseling and Testing
WHO	World Health Organization
ZDV	Zidovudine

1. Introduction

1.1 Global Epidemiology of HIV

The epidemiological trend of human immunodeficiency virus (HIV) in 2016 is encouraging, with the decline of death from HIV/AIDS by 40% since 2000 [1, 2]. According to the UNAIDS global report at the end of 2014, 36.9 million people were living with HIV, 2 million people were newly infected, and 1.2 million had died from HIV related causes. Since 1990, this is the lowest number of new infections that the world has experienced in 20 years. Most importantly, the numbers of 15-24 years olds that are acquiring HIV infection have declined from 980,000 (930,000-1,020,000) in 2000 to 620,000 in 2014 [1]. This decline is largely attributed to Sub Saharan Africa (SSA) where 41% reduction was seen in new HIV infections from 2000-2014 [1].

Sub-Sahara Africa is the hardest hit region by the epidemic and by the end of 2014 , 25.8 million people (70%) were living with HIV of which 2.3 million were children < 15 years of age and 58% were women [2- 4] .

In 2014, 1.4 million newly infected adults and children resided in SSA [2]. Children <15 years old accounted for an estimate 190,000 of the new infections and 130,000 deaths in 2014 [2]. There are regional variations in the severity of the epidemic within Sub- Sharan Africa [5]. The worst affected sub region is Southern Africa countries with the highest HIV prevalence among 15-49 age adults are Swaziland (31%), Lesotho (26.7%), Zimbabwe (17.7%), South Africa (14.1%), Mozambique (13.1%) and Malawi (12.9%) [5].

Data from WHO Africa Region 2013 indicate that women were twice more likely to be infected than men in all Sub-Sahara African countries [5], and acquire HIV infection 5-7 years earlier than man [4]. Out of the 2.8 million, aged 15-24 that are living with HIV 63% are women [4]. Sex differences in HIV prevalence being both biologically and socially determined as follows: the exposure of the mucosal lining of the vagina to the concentration of HIV in the semen, cervical ectopy in young girls, gender associated violence, economic empowerment, sexual and reproductive health decision making [6,7].

Reduction of HIV incidence and HIV related mortality rates are mainly due to scale-up of antiretroviral therapy (ART). ART in 2014 was accessed by 10.7 million people as compared to 100,000 in 2002 [1]. Furthermore, reductions have been attributed to several prevention

strategies: behavioral change programs, condoms, HIV Testing, blood supply safety, harm reduction efforts for injecting drug users, male circumcision, PMTCT programs, ART treatment, pre-exposure antiretroviral prophylaxis (PrEP) for individuals at high risk for HIV infection [1].

Global support for the fight against HIV/AIDS has resulted through a multilateral funding mechanism and government bilateral support that built a very strong momentum and commitment; key players are the Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund), President's Plan for Emergency AIDS Relief (PEPFAR), the Bill and Melinda Gates Foundation, and the Clinton Foundation. The Global Fund is one of the largest funders for HIV, TB and Malaria programs in low and middle income countries, investing 4 billion year to support national and community programs [1]. PEPFAR 2003-2014 has funded 52 billion towards HIV Programs and also contributes to the Global Fund. Domestic funding have also increased since 2000 and in 2014, domestic funding in comprised of 57% of all funding for HIV programs [1]. Domestic funding is important in strengthening the ownership and sustainability of HIV programs at national level.

1.2 Prevention of Mother to Child Transmission

In June 1983 there were first reports of possible mother to child transmission seen in 21 infants [9]. Since then numerous cohort studies looked at risk factors that can be associated with mother-to-child transmission of HIV and the development of prevention interventions [10-20] resulting into three prevention: 1) reducing maternal viral load with ART; 2) prevention of maternal virus at birth through less traumatic obstetric methods (e.g. caesarian sections) 3) avoidance of breastfeeding. These research findings were rapidly translated into policy and practice. The fist PMTCT intervention that was made into policy in 1985 by CDC was HIV infected women shouldn't breastfeed but use formula feeding instead [21]. In 1994 the landmark PACTG 076 study demonstrated a 67% reduction in perinatal HIV transmission with the administration of a combination of prenatal, intrapartum and neonatal zidovudine [22]. This regimen was rapidly included in the US Public Health Service Guidelines and implemented all over the US [23]. In 1999 the results European Mode of Delivery trial showed that the rate of MTCT further decreased to 1.8% if a women who received ZDV prophylaxis was also randomized to deliver in elective cesarean section versus women who delivered vaginally (10.5%) [24]. PACTG 076 and the European Mode of Delivery results furthers decreased mother to child transmission rate in

US and Europe but these regimen were not implemented in low and middle income countries due to cost and feasibility [25].

In an effort to finding a shorter, more feasible, and less expensive ARV treatment in prevention mother to child transmission researchers initiated a series of cohort studies in the Americas, Europe, Africa, and Asia which eventually translated to policy and WHO Guidelines for prevention of mother to child transmission in low and middle income countries [27-32].

1.3 Evolution of WHO PMTCT Guidelines

The WHO PMTCT Guidelines have been revised 5 times in 13 years based on new evidence from clinical trials, program feasibility, and cost implications [33-37]. The first WHO Guideline in 2001 *"Prevention of Mother to Child Transmission of HIV: Selection and Use of Nevirapine"* was influenced by the HIVNET 012 trial in Uganda [30, 31, 33] which compared single dose Nevirapine to the mother at the time of labor and to the infants within 72 hours of birth versus ZDV given to the mother during labor and to infants for seven days. The mother to child transmission rate in the NVP group at 6-8 weeks was 8.2% and 10.1% at 18 month while in the ZDV group transmission rate were 20% and 25.8%, respectively [30,31]. Following the favorable outcome in the NVP groups the Guideline recommended a single oral NVP 200mg dose tablet to be taken by the mother at the onset of labor and an oral dose of NVP in suspension (2mg/kg) to be given to the newborn within 72 hours of birth (33).

The efficacy of ZDV plus single dose maternal and infant nevirapine has been examined in several clinical trials [26-29, 38]. The Perinatal and HIV Prevention Trial in Thailand in 2004 showed that a single dose of NVP combined with oral ZDV starting at 28 weeks of gestation to the mother and a one week ZDV to the infant reduced mother to child transmission rate to 2% [32]. WHO PMTCT Guideline in 2004, adopted this finding and recommended for HIV pregnant women with CD4 count < 200cells/µL to initiate triple ART including ZDV, Lamivudine (3TC) +NVP or Stavudine (d4T) +3TC+NVP [34]. Infants that are born to mothers either receiving 1st line or 2nd line regimen should be given ZDV, sdNVP, or sdNVP +ZDV for one week. HIV infected pregnant mothers who were not in need of ART for their health were recommended to start ZDV from 28 weeks until labor, and a sdNVP at the onset of labor, while the infants were treated with single dose NVP plus ZDV for one week [34].

The third WHO PMTCT Guideline 2006 recommended for women in need for triple ART for their own health ZDV+ 3TC+ NVP and for infants ZDV for seven days except if the mother receives less than four weeks of ART. In this case infant ZDV prophylaxis was to be given for four weeks. Pregnant women not eligible for ART were recommended to start dual ARV prophylaxis of ZDV and 3TC from weeks 28 plus sdNVP during delivery [35].

Table 1: Prophylactic Regimen for Pregnant Who do need ART for their own

Antepartum	AZT 300mg twice a day starting at 26 weeks or as soon as possible
Intrapartum	sd-NVP +AZT/3TC
Post- Partum	AZT/3TC for 7 days
Infants	Irrespective of mode of infant feeding sdNVP within 72 hours of delivery + AZT for 7 days

The Kesho Bora Study findings strongly influenced the 2010 WHO PMTCT Guideline [39, 40] and compared for the first the safety and efficacy of triple ART (ZDV+ Lamivudine + Lopinavir/Ritonavir) from the last trimester of pregnancy, continued during breastfeeding , up to 6 month of age against WHO 2004 PMTCT recommended regimen [39,40]. The mother to child transmission and the infant mortality rate following the WHO 2006 procedures at 12 month was 9.5% and 6.5%, respectively, as compared to 5.4% and 4.8% from the intervention [39, 40]. These results led to the following implications: 1) initiation of ART early in pregnancy or even before pregnancy 2) 80% transmission occur in women with CD4 counts < 350 cell/µ 3) priority access to ARV for HIV positive women who plan to get pregnant [39,40]. The WHO 2010 PMTCT recommendation resulting from the Kesho Bora study findings are presented in table [36]. The recommendation included an option based on maternal single ZDV dose prophylactic treatment for those not in needs for ART for their own health (Option A) and a triple ART approach for mothers eligible for ART by CD4 counts (Option B).

Table 2: WHO 2010 PMTCT Guidelines

Option A	Option B
Mother	**Mother**
Antepartum: AZT from 14 weeks of gestation and continued during pregnancy	Triple ARV prophylaxis from 14 weeks of gestation or as soon as feasible
Intrapartum: sd-NVP +AZT/3TC	Triple ARV prophylaxis
Postpartum: AZT/3TC for 7 days	Triple ARV prophylaxis continued until 1 week after infant exposure to breastmilk has ended
Infant	**Infant**
For breastfeeding infants: Daily NVP for a minimum of 4-6 weeks, and until 1 week after exposure to breast milk has ended	*Irrespective of mode of Infant feeding.* Daily NVP or twice daily AZT from birth until 4 to 6 weeks of age.
Infants receiving replacement feeding only daily NVP or sd-NVP+ twice –daily AZT until 4-6weeks of age.	

In 2011, the Malawian Ministry of Health adopted a pragmatic and ambitious public health approach to improve the low PMTCT coverage in Malawi known as "Option B+," which provides all HIV-infected pregnant and breastfeeding women with immediate, lifelong ART, regardless of clinical stage or CD4 cell count [41]. By eliminating the complex 2010 PMTCT algorithms, delays from CD4 testing, and ART re-initiations with each pregnancy with Option B, Option B+ simplified the PMTCT process, with expected important benefits for HIV-infected women, their HIV-exposed infants, and their HIV-uninfected sex partners . This translated into the final WHO Option B+ PMTCT Guideline for both ART and PMTCT in 2013 [37, 42]. Even though Option A and Option B in the 2010 Guideline had similar efficacy in clinical trial settings it has been difficult to scale up Option A in low income countries (37, 42). Option B+ was meanwhile adopted by most African countries because it is given to all pregnant women and continued during pregnancy, labor, and postpartum, the fixed dose regimen TDF+ 3TC+EFV can be given to both pregnant and non-pregnant adults, CD4 count is not a requirement for initiation, Option B+ delays maternal disease progression, it maximize ART coverage and decrease mother to child

transmission, and reduces sexual transmission of HIV to sero-discordant partner (37, 41). In the Malawi study where the cost effectiveness of Option A, Option B and Option B+ was analyzed it was shown that Option B+ represented a cost effective strategy because it prevented new HIV infections but also improved the survival of infants. [43]

WHO PMTCT Guideline regimens in research clinical trial settings show impressive efficacy but translation of these regimens into implementation in low and middle income countries has remained a challenge [25, 44]

1.4 PMTCT Update in Sub-Sharan Africa

PMTCT programs have been implemented in Sub Saharan Africa since early 2000 but the number of newly infected children has only declined by 24% between 2000- 2009 (8). Despite the clinical trial findings on efficacious regimens and major initiatives launched to make treatment accessible through WHO, GFTAM, PEPFAR, Clinton Foundation and Bill and Melinda Gates Foundation, PMTCT programs in SSA have lagged behind [25,44]. Reasons for this are low uptake of HIV testing, weak health infrastructures, weak support from the highest level of government and public health authorities, home delivery, lack of male involvement, lack of family planning, limited donor funding, and PMTCT drug and HIV kit stock outs [25, 44].

Overall there has been progress between 2000-2015 in reduction of new HIV infection in children and ART coverage for HIV positive women. At the same time there have been challenges in the reduction of mother to child transmission, timely early infant diagnosis of HIV, ART coverage for children, decreasing the incidence of new HIV infection among women within child bearing age, and family planning [8]

The Global Plan towards the elimination of new HIV infections among children and keeping mothers alive in 2011 was launched with key decision makers from 35 countries at the UN to address these limitations of PMTCT programs in 21 low and middle income countries which account for 90% of the HIV positive children [8].

The two main targets of the Global Plan are:

1. To reduce the number of new childhood infections by 90% by

   I. Reducing AIDS related infant death by >50%.

   II. providing ARV for all HIV infected Children

2. Reduce the number of HIV related maternal death by 50%.

These targets and indicators were each then combined within four WHO pronged approaches for PMTCT to create a scaled up and improved PMTCT program with targets that can be monitored [8]. The Global Plan main target was to reduce the number of new childhood infections by 90% by reducing AIDS related infant death by >50% and by providing ARV for all HIV infected children [8]. Since the launch of the Global Plan in 2011, HIV incidence in children among the 21 priority countries declined by 76% in South Africa, 60% in Ethiopia , Mozambique, Namibia, Swaziland, Uganda, and Tanzania, 40% in Botswana, Ghana, Lesotho, Malawi and Zimbabwe [1, 8]. In spite of this progress the identification and ARV treatment of HIV positive infants is lagging. WHO recommends that early infant diagnosis is performed within 4-6 weeks after birth and the dry blood spot results be returned to the health facility sites within 2 month of the infant birth date [45]. In most SSA countries Dry blood spots are taken from the infant at health centers and sent to a regional or referral laboratory. At the laboratory, the dried blood spots on the cards are analyzed using DNA- PCR technology. The results are then sent back to all the different health centers through a motorcycle courier or SMS. The cascade of events that have to take place before the DBS reaches the health facility and linkage of the infant to treatment is error prone. The nature of the cascade can have a long waiting period which causes mothers not to come back to health facility, delayed initiation to ARV for infant or even failure to initiate treatment for infant.

In 2014, only 49% of infants in the 21 priority countries had EID testing done within a two month period [8]. In a study from South Africa missed opportunities for early infant diagnosis included identification of HIV exposed infants by health providers (mothers had to carry Infant Health to Chart /booklet for identification), MTCT knowledge, adherence to treatment, and discrimination as the main determinant for uptake of EID services [46]. A study in rural Kenya indicated that mothers didn't know the exact time points, or type of tests required for EID

service [47]. Another study in Uganda found that the average age of infant for first EID was 14 weeks and the turnaround time of EID results on average was 45 days and 29.5 % of care takers didn't come back for test results [48]. In the same study for second EID test for diagnosis of MTCT at 6 month only 39.7 % brought their children back for testing and only 40% of those that were tested positive was initiated on ART [48].

In an Ethiopian study reasons for mothers not coming back after the end of breast feeding period for EID was the lack of understanding that mother to child transmission can still take place after breastfeeding period even if the 6 weeks DBS result was negative [49]. The failure to identify HIV positive infants through EID and initiate ART timely causes high rate of mortality, development of immunodeficiency syndrome, encephalopathy and neurodevelopmental effects [50-52]. Among the 21 priority countries only 31% of children are receiving ARV's [8]. The highest provision of ARV's for children among the priority countries are Namibia with 66%, Botswana with 53% and South Africa with 49% [8]. The lowest provision of ARV's to children in the 21 Priority countries are Chad 0%, Cameroon 11 % and Nigeria 12% [8]. The reasons for low ARV provision to children are the availability of children friendly ARV's, low a rate of pediatric HIV case finding , poor linkage to care and treatment, and knowledge of ARV's by heath care giver [8]. Similar pattern are seen for recommended cotrimoxazole provision to HIV exposed infants; only 8 countries provided cotrimoxazole for 50% of HIV exposed infants [8]. In an Ethiopian study predictors for HIV infected infant mortality was not receiving cotrimoxazole at baseline, low hemoglobin, absolute CD4 count, and delayed initiation of ART [53]. These studies indicate that there are challenges for identifying infants, and enrolling them in an ART program and dispensing cotrimoxazole in a timely manner.

Another way of reducing infection in infants is by preventing of infection among women of childbearing age. The high incidence of HIV among 15-24 years old women in SSA Africa are attributed to gender inequality in the form of intimate partner violence , coerced sex among adolescent girls, age of sexual debut, relationship with older men partners and economic inequity [54-57]. Between 2009 and 2014 at total of 3.8 million women in the child bearing age group acquired HIV in the 21 priority countries [8]. The decline of new infection in women in reproductive age was only 15% between 2000 and 2009 (8). PMTCT programs need to engage male partners, but mainly biomedical and behavioral interventions have to be designed

specifically to this group of young women to target this hidden epidemic within the generalized epidemic [58, 59].

The Global Plan also recommends reducing MTCT to 5% by providing 90% of pregnant women in need of ARV for their own health. Presently, Option B+ is now being implemented since 2013 in most the 21 priority countries (except Cote d'Ivoire, Ghana and Nigeria) [8]. Option B+ is a fixed dose of ART TDF+ 3TC + EFV which is offered to HIV positive mothers for life long and it is not dependent on CD4 count or WHO stage [37]. The impact of adherence to ART is apparent when looking at the final HIV transmission rate after the end of breastfeeding period [8]. Prior to the launch of the Global Plan transmission rates at 6 weeks and at the end of breastfeeding altogether in 2009 were 28%, after the launch of the Global Plan MTCT was halved to 14% except for Chad and DRC which have a MTCT transmission rate of 34% and 31% [8]. Studies that have looked at Option B+ and adherence indicate the main reasons for not adhering were side effects (which included dizziness, nausea, nightmares, hallucinations), and concerns over partner's acceptance of treatment, feeling healthy (not needing ART), economic concerns and food security [60-62]

Significant predictors for adherence to Option B+ were adequate time and support for women to make decision, consistent pre-ART counseling , early support for patients experiencing side effects, and effort to bring women who stop ART treatment back through mother support groups and adherence clubs [60,61]. In the 21 priority countries 17 countries have transitioned to lifelong treatment Option B+ and ARV coverage has increased from 37% in 2009 to 77% in 2014 [8]. In seven countries Botswana, Mozambique, Namibia, South Africa, Swaziland and Uganda and Tanzania the coverage for ARV for pregnant women living with HIV has increased to 90% [8].

1.5 Ethiopia and HIV Response

1.5.1 Epidemiology:

Ethiopia is the second-largest country in Africa, which has an estimated population of nearly 94,101,000 [63] and a growth rate of 2.6 % [64] per year. Ethiopia is a predominantly rural and young society, with 83.9 % percent living in the rural and 19% living in urban areas. The proportion of the population under age 15 is 45 %, with only 3.2 percent is above age 65 [64].

Ethiopia's Gross National income (GNI) is 1350 USD and is classified as a Low income country by the World Bank [63]. Ethiopia is divided into 9 regions Afar, Amhara, Benishangul-Gumuz, Gambella, Harari, Oromia, Somali, Southern Nation Nationalities and People Region (SNNP), Tigray and two City Administration's Addis Ababa, and Dire Dawa.

Education is a key factor in health outcomes and has a strong effect on reproductive health, fertility, infant and child mortality and morbidity. In Ethiopia, overall 51% women and 30% of men have no formal education, in the age group 15-49, 38% of women and 67% men are literate (DHS 2011) [64]. The total fertility rate is 4.8 children per household, women in urban areas have 2.6 children as compared with 5.5 children in rural areas [64].

According to Ethiopia MDG Report in 1990 maternal mortality was 871/ 100,000, infant mortality was 123/1000, less than five mortality was 166.2/1000 and neonatal deaths were 54/1000 [65]. Due to this major health system revitalization Ethiopia has made significant progress on the MDG Goal 4 in 2013, infant mortality rates have declined to 88/1000 births , under 5 mortality rates have declined to 60/1000 live births [65]. However the observed progress in reducing infant under 5 years mortality is not uniform between urban/ rural and geographic location [65].

Neonatal death have also declined to 37/1000 live births in 2011 but the reduction is minimal and still contributes significantly to childhood deaths [64, 67]. Ethiopia has increased primary health coverage to 94% by increasing the number of health posts, health centers and the number of hospitals [65]. Ethiopia has also increased full immunization coverage to 82.9% but only Addis Ababa and SNNP showed high immunization coverage while the other regions had lower immunization performance than the national coverage [65].

Of all the MDG Goals reduction of maternal mortality the MDG Goal 4 was the slowest from 1400/100,000 in 1990, to 420 in 2013, because of delayed utilization of skilled care, limited availability of emergency obstetric and newborn care, and low post-partum visit [65, 68]

1.5.2 Health System and Health Policy:

The health sector has introduced a three-tier health care delivery system [69, 70]. Level 1 comprises of a primary hospital (to cover 60,000-100,000 people), health centers (1/15,000-25,000 population) and their satellite Health Posts (1/3,000-5,000 population) connected to each other by a referral system. The health center and health posts form a Primary Health Care Unit (PHCU) Level 2 is made of General Hospital covering a population of 1-1.5 million people; and Level 3 are Specialized Hospital covering a population of 3.5-5 million people [69, 70].

With the decentralized government structure, decision making for public service delivery is under the authority of the regions and districts. The Woreda (District) Health Offices manage and coordinate the operation of the district health system under their jurisdiction. The Woreda Health Offices use the Health Sector Development Programs (HSDP) as the basis for developing the annual plan at all level [69, 70].

Following the change of government in 1991, the new Government of Ethiopia first national health policy, which was followed by the formulation of consecutive phases of comprehensive Health Sector Development Programs (HSDPs) forming a twenty year strategic roadmap for the health sector. Health Sector Development Programs (HSDPs) are designed to take in account national health policy, strategies and prevailing national health problems and emerging health problems in the country [69, 70].

HDSP I: 1997/98- 2002

HSDP II: 2003-2005

HSDP III: 2005/6 -2009/2010

HSDP IV: 2010/11 – 2014/15

The current HSDP IV 2010/11 to 2014/15 main strategic directions are: strengthen health extension program (HEP), improving quality of health care, scaling up civil service reform, focused attention on maternal health, TB, and PMTCT program's. Other HSDP IV focus areas are health resource development, health infrastructure, addition support to regions that lag on targets, climate change and disparities on health and gender [71].

1.5.3 HIV Epidemic

The HIV/ AIDS epidemic in Ethiopia, similar to most Sub Sahara African countries, began in the late 1970 or early 1980's [72]. The first HIV positive patient was reported by Lester et al in 1986 among hospitalized patients at a hospital in Black Lion Hospital, Addis Ababa [73]. HIV/AIDS was also detected among 5,565 military recruits showing a prevalence of 0.07 % [74]. The epidemic was mainly concentrated in urban areas among most at risk population (MARP) such as commercial sex workers (17%) who resided along the main trading routes, truck drivers (13%) [75-77]. A 1990 papers indicated that HIV prevalence was increasing substantially in commercial sex workers in Addis Ababa [76-77]. In 1985 the Department of AIDS that consisted of the National Task Force on the Prevention and Control of HIV and AIDS and the National AIDS Control Program was established [78]. This facilitated the drafting of the first AIDS Policy, the establishment of a surveillance system, and initiation of HIV/ AIDS education for the most at risk population [78].

In 1991 the Dergue government was overthrown and Ethiopian People's Revolutionary Democratic Front (EPRDF) took office. The 30 year civil war that culminated with an EPDRF victory exacted heavy tolls on the whole country [79]. A large number of hospitals in the northern part of the country where the war was taking place had been destroyed or was not functioning. (86) In 1990 coverage of health facilities was estimated at 45%, only 16% of mothers attended ANC, 5% of deliveries were assisted by health personnel, EPI coverage was 26% for mothers and 13% for children, CPR was 4%, and only 19% of people had access to safe water [79].

In 1999 the new government issued a 5-year Multi Sectoral HIV/AIDS Strategic Plan for 2000-2004. The objective of the strategic plan is to reduce HIV transmission and HIV/AIDS morbidity and mortality [80]. HIV prevention mainly centered on behavior change on safer sex messages using condoms, and radio programs directed to the youth, community based methods such as drama, art, and peer education [81]. Voluntary counseling and Testing (VCT) services were scarce between 1995-2000 and only 1.0% of people in rural areas and 9.3% in towns reported on being tested but 64% of rural and 69.4% of urban respondents wanted to be tested indicating high levels of unmet demand [81].

The mid 1980's to 2000 can be characterized as very limited funding, implementation of HIV programs was low, HIV/AIDS policy was just issued and there was no guidelines or training materials for HIV, tuberculosis (TB), sexually transmitted disease (STI), blood safety, VCT, PMTCT, ART, opportunistic infections (OI surveillance), and monitoring and evaluation (M&E) [78].

In 2001 MOH issued guidelines for ARV treatment, VCT, a handbook for HIV/AIDS home care and a policy for ARV supply and use [82-85]. The ART program was launched in July 2003 for only those who can pay and the cost was between 30-90 USD per month depending on the ART selected [81]. Diagnostic lab test for CD4 was also available for only those who can afford to pay 36- 80 USD [81]. The ARV was dispensed only from the Ethiopian Red Cross Pharmacy and a Municipality Pharmacy. At the end of 2004, 9,700 people were on treatment nationally [81].

In 2003, the adult HIV incidence in the urban area was 1.82% while for the rural it was 0.46% [86]. The estimated national adult prevalence was 4.4% of which 12.6% was urban and 2.6% was rural. The cumulative number of people living with virus was 1.5 million (3.8% males and 5% female, 12.6% urban, 2.6% rural), 96,000 were children >15 years of age. The estimated new HIV case adult population was 98,000 (46% male and 54% female), 245,000 PLWHA were in need of ART, 90,000 adults and 25,000 children died from HIV/AIDS related causes. At the end of 2003 there was an estimated 539,000 AIDS orphans [86]

In January 2005 the government launched a free ART program and by the end of July 2006, 45,595 patients were started on ART at 132 facilities. Of the clients starting on ART 47% were adult males, 48% were adult females, and 4% were children. The national adult prevalence decreased to 3.5% and it was estimated 1.32 million people were living with HIV AIDS, and there was 128,900 new infections altogether of which 30,300 were infants [87].

Since 2005 HIV incidence and AIDS mortality have declined according to the last Ethiopian Demographic Health Survey 2011 the adult HIV prevalence is 1.5% [64]. However prevalence varies according to age, sex, gender and regional location [64]. The HIV prevalence rates in woman were twice as high compared to males (1.9% versus 1%) [64]. The distribution of HIV prevalence also varied by age peaking earlier in female's 30-34 age groups as compared to 35-39 in males. [64] The HIV epidemic is concentrated in urban areas with 4.2% prevalence as

compared to 0.6% prevalence in rural areas. HIV prevalence is highest in the Gambella region, Addis Ababa, and Dire Dawa and it is lowest in SNNPR and Oromia have the lowest prevalence [64].

Antenatal sentinel surveillance in 2011 indicates that HIV prevalence among pregnant woman aged between15-49 has declined both in urban and rural areas [64]. HIV prevalence in urban areas for women between15-49 declines from 14.3% in 2001 to 4.4% in 2012 and in the rural areas it declined from 4.1% in 2003 to 1.8% in 2012 [64].

High risk population are diverse and not well delineated but past studies have shown female sex workers, seasonal mobile workers, university and high school students, uniformed services, prisoners, truck drivers, and sero-discordant couples may be at higher risk for HIV infection [88].

The main reasons for the decline of HIV in Ethiopia are: 1) rapid expansion of HIV testing and counselling and behavioral change programs through the Keble (the smallest administrative unit equivalent to a ward or neighborhood), involvement of Health Extension Workers at community grass root level, rapid expansion of ART coverage, high rates of male circumcision (92%), low rates of pre-marital sex and low rates of extramarital sex. [99]

1.5.4 PMTCT

PMTCT guideline was published in 2001 but facility based service delivery was initiated in 13 pilot health centers on Feb 2004 (program launched September 2003) [89]. The Hareg Prevention of Mother-to-Child Transmission (PMTCT) Project was funded by the U.S. Government's and the treatment was based on WHO Guideline 2001 Data from the first 13 sites indicate only 55% who were pretested were counselled, there were 627 pregnant women who tested positive, 221 women and 161 infants who took NVP [89]. In 2005, FMOH AIDS 6th report indicates that a total of 52,428 pregnant women were tested for HIV, 4,172 tested HIV positive, of these 2,208 (52.9%) of the pregnant women and 1341 (32%) of their infants received nevirapine for PMTCT [87]. Facility based PMTCT services start increasing nationally from 32 facilities in 2004 to 129 facilities in 2005, 2012 there were 1901 facilities with PMTCT services [88].

The first national PMTCT guidelines in Ethiopia were developed in 2001, but were only implemented in 2003 in limited public health facilities. The 2001 National PMTCT Guideline was in line with WHO 2001 recommendation of sdNVP [91]. Due to lack of free access for ARV prophylaxis the implementation of the basic PMTCT package remained a challenge until 2005 [87]. The 2001 National PMTCT Guideline was revised in 2007 and this incorporated WHO PMTCT 2004 and 2006 revisions which were HIV counseling and testing from the opt-in to the opt-out policy, initiation of first line regime AZT+ 3TC+ NVP for mothers with CD4 <200 cells/µL, and women who were not eligible for ARV's should initiate AZT starting at 28 week [92]. The 2007 National PMTCT guideline was revised again in 2011 in line with the WHO recommendations 2010 of Option A [93]. The two key revisions from WHO PMTCT 2010 that were incorporated into the new 2011 National PMTCT Guidelines are the increase of CD4 count from <200 to <350 as cut off point for ART initiation and ARV prophylaxis for women who are not in need of ART for their own health can start ARV as early as 14 weeks of gestation [93]. Simultaneously, in 2011 Federal Ministry of Health (FMOH) came up with the new Accelerated PMTCT strategy and coordination plan [94]. The FMOH set ambitious PMTCT national targets for 2015 in the Fourth Health Sector Development Program, to provide ANC service to 90% of pregnant women, to ensure all women are attended at delivery of which at least 62% should be cared for by skilled health attendants and 38% health access workers and lastly to provide ARV prophylaxis to 80% of HIV positive pregnant women [94]. In 2013 the National PMTCT Guideline was revised to the Option B+ after Ethiopia signed on to *The Global Plan towards the Elimination Of new HIV infections among Children by 2015 and keeping their mother alive* 2011 [95]. In 2013, FMOH Revised the PMTCT guideline to WHO 2013 Option B+ regimen and also developed MTCT Elimination Plan (*eMTCT*) with the goal to provide Option B+ to 95% of HIV positive pregnant women, reduce new infections among reproductive women by 50%,and reduce unmet need by of family planning by 10% and reduce MTCT to less than 5% [96,97].

1.5.5 Antenatal care as entry point to PMTCT

Antenatal care during pregnancy is the first intervention in order to maximize the chances of preventing mother-to-child transmission. Nigatu et al. have analyzed national PMTCT data from 2004 - 2009 and the antenatal care service coverage for at least one visit during pregnancy increased from 50% in 2006 to 71% in 2010 [98]. The same study also looked at PMTCT services

at ANC clinics between 2006 and 2010, reporting an increase from 171 PMTCT service sites in 2006 to 1352 sites in 2010. Paradoxically, the expansion of PMTCT sites didn't coincide with an increase in mothers receiving PMTCT services at ANC clinics. According to the 2006-2010 national PMTCT data report 1.3 million pregnant mothers that attended ANC sites were not provided PMTCT service [98].

In 2013 the total number of health facilities that provided PMTCT services has increased to 2,150 which mean 64.4% of public hospitals and health centers provide PMTCT services. Among the 2.3 million pregnancies in 2013 80% of the expectant mothers attended ANC [99].

Counseling, HIV testing at antenatal sites with PMTCT services generally faces challenges for obvious reasons of client's fear of result, concerns of confidentiality, fear of disclosing results to partners, and fear of discrimination [100]. Nevertheless, acceptance of HIV testing has dramatically increased Ethiopia between 2006- 2010, the number of women that have accepted counseling and testing rose from 57% in 2006 to 92% in 2010 [98]. The increase is partially due to the fact HIV Testing and Counseling guideline changed in 2008 from the "opt –in' approach to the "opt out" approach [101]

Despite the significant progress, 300,000 mothers who received counseling service did not receive HIV testing at antenatal clinics from 2006 to 2010. According to Nigatu et al. in 2010, an average of 326 women present for ANC every work day missed HIV counseling services. However the number the of HIV positive women that have not been counseled at ANC sites has decreased from 230,000 in 2006 to 85,210 in 2010 [98].

National PMTCT data analysis in Addis Ababa between 2004- 2009 indicates that 663,603 pregnant women attended ANC and 24.6% was new attendees, overall 135, 196 women and 6,664 partners received HIV counseling and 6.2% (8,467) were HIV positive [101].

The Multi-Sectoral HIV response report for 2012/2013 indicates that there still remain missed opportunities for HIV Counseling and Testing. In 2013 there were 2.9 million expected pregnancies and 80% pregnant mothers attended ANC but only 70% of them were counseled and tested [99].

1.5.6 ARVs as essential component of PMTCT in Ethiopia

Free access to ARV prophylaxis became available in 2005 in Ethiopia [87] National ARV coverage for mothers and infants is one of the major challenges that Ethiopian is still PMTCT program is facing in 2015 (8) .

A five year national level PMTCT data analysis (2006-2010) by Nigatu et al. revealed that 56% of women received ARV's, while 44% of pregnant HIV positive mothers didn't receive ARV prophylaxis [98]. Similar trend is observed in the analysis of monthly PMTCT reports from Addis Ababa between 2004 and 2009, ARV prophylaxis uptake was 42.4% for mothers and 31.08% for infants in cohort of 8,467 HIV positive pregnant women [101].

In a 2010 cohort study in Addis Ababa that looked at 282 HIV positive pregnant women, 232 initiated medications during pregnancy, 154 initiated ZDV prophylaxes and 78 lifelong ART. Among the 232 women that initiated medication during pregnancy, only 171 ingested medication during labor, and this was strongly associated to giving birth at health facility (OR 13.64, 95% CI 4.64—40.12) [102].

A PEPFAR data analysis from 2013 indicated that 17,742 women were found to be HIV positive and only 75% of these identified received ARV and even fewer of their infants received ARV prophylaxis [99]. The Global Plan 2015 also showed that in 2013 in Ethiopia there was only 16% ARV Coverage for children and in 2014 the coverage increased to only 22% [8]. In spite of challenges faced at every step of the PMTCT cascade, the number of HIV positive pregnant women who were identified, took ARV has increased by several folds between 2006- 2013 [99].

1.5.7 Early Infant Diagnosis and Mother to Child Transmission rate

WHO recommends that infants exposed to HIV should be tested between 4 and6 weeks and if infected should start treatment immediately [45]. Infants infected in utero, during labor or deliveries have a poor prognosis compared to those infected during breastfeeding, they require urgent ARV to prevent early mortality [103].

The PMTCT national data analysis from 2006-2010 by Nigatu et al. showed that 56% of HIV exposed infants did not receive ARV prophylaxis [98]. Similar trend were observed by a 2011 retrospective study done by Mulatu et al. which analyzed data from 2008-2009 in Zeweditu

Memorial and Yekatit Hospital from 118 HIV exposed infants [49]. The study showed the ARV uptake at birth was 91%, but eventually 31% of the infants were lost to follow up and their cotrimoxazole treatment interrupted [49].

In the cohort study by Mirkuzie in 2009 out of the 221 live births from HIV positive mothers, 115 infants received their first EID diagnosis but only 71 received documented results. The mother to child transmission rate was approximately between 8.2 % and 9.5% [102]. The main reasons for not having documented EID test results were : 1) results were not collected from the central laboratory, 2) misplacing of results at the facilities, 3) DBS not collected from infants due to lack of training [102].

Follow up on HIV testing on infants also faced challenges as reported by another retrospective study by Mirkuzie et al [101]. From 2004-2009 only 896 (10.6%) infants completed the follow up HIV testing, of which 106 infants were found to be positive. The mother to child transmission rate calculated for the cohort of infants in 2006 that were on sdNVP regimen tested at ≥ 18 month was 14.3%, and the MTCT rate for infants in 2007 was 14.9% ,and in 2009 the MTCT rate was 8.2% , this decline may be due to guideline change in 2008 to the more efficacious ZDV regimen [101]

The major determinants of mother to child transmission in Ethiopia have been discussed in two retrospective follow up studies that were done in Ethiopia at Gondar University Referral Hospital from 2005-2011 and Dil Chora Referral Hospital from 2005-2013. (104,105) Both retrospective studies had similar findings on the major predictors to mother to child transmission, which were delayed infant linkage to care, rural dwelling, delivery at home, mixed feeding, infant not receiving ARV prophylaxis at birth, mother infant pair not on ARV [104,105]. In Gondar University Hospital the MTCT rate was 10% and at Dil Chora Hospital it was 15.7% [104,105].

The Global Plan Progress report for 2015 for Ethiopia indicates the number of new infections among children between 0-14 years old in 2013 and 2014 were 7000 and 4800, the MTCT rate at 6 weeks was 8% and 6%, and at the end of the breastfeeding period increased to 30% and 26%, respectively [8].

Nevertheless, even if the infant is successfully tested and linked to the health system, sustainability of access to treatment and care of children remains a challenge. The ARV coverage of children in Africa according to the 2015 Global Plan update on HIV in the WHO Region is estimated at 31% and in 2011 it was 26% which shows that there hasn't been any progress [8]. A qualitative study by Baidgilin et al. on the barriers to ARV adherence among HIV infected pediatric patients that was conducted in 6 selected hospitals in Addis Abeba indicated 1) heavy pill burden, 2) stigma and discrimination, 3) cost and access to transportation, 4) economic problems at the house, 5) lack of nutritional support, 6) lack of medication understanding – as barriers for ARV adherence [106].

In 2015 out of the 22,000 children infected through mother to child transmission 190,000 children resided in Africa, although vertical HIV transmission could easily be prevented [1]. In high income countries, peri-natal transmission is less than 1% due to caesarian sections and effective uptake of antiretroviral therapy (ARV) by HIV positive pregnant women [25]. In low and middle income countries PMTCT coverage is low and in consequence transmission rates remain high [25, 44].

1.6 Summary

Although the advance in PMTCT regimen suitable for low income regions such as Sub-Saharan Africa implementation of these findings have been slow compared with the rapid implementation of these findings in the mid 1990's in high income countries and the decline of MTCT presently to 1-2%. This is despite major initiatives such as GAFTM, PEPFAR, Bill Melinda Gates Foundation and Clinton Foundation who have made treatment access a reality to millions of people in SSA. Even though there are many factors for this slow progress, the main factor is political will and weak health infrastructure. A testimonial for political will is Cuba which became the first country to formally validate the elimination of MTCT [8]. Botswana and Thailand are also good examples of how the political will of the government has been able to reduce MTCT below 5% [25]. It is important for PMTCT programs in low income countries adopt factors that made PMTCT a success from other countries but also address factors for failures within their own programs in order to achieve better result.

1.6.1 Problem statement:

Ethiopia PMTCT program is relatively young, approximately 10 years (2005-2015), since the complete basic PMTCT package has been rolled out nationally; there has been impressive progress in this short time frame. This study looked at PMTCT intervention outcomes such as ART regimens, obstetric practices, breastfeeding, and linkages of HIV positive infants to HIV care and treatment at 7 health centers in Addis Ababa. Even though the study results cannot be strictly generalized to all the health centers in urban Ethiopia, the findings of this study may assist to improve PMTCT care services in connection to the new 2013 Nation MTCT Elimination Plan (eMTCT) with the goal to provide Option B+ to 95% of HIV positive pregnant women, reduce new infections among reproductive women by 50%,and reduce unmet need by of family planning by 10% and reduce MTCT to less than 5% [96].

1.7 Objectives

The general objective of the study was to assess PMTCT uptake level and the subsequent linkage of infants to HIV care and treatment services.

The specific objectives were:

1. To assess adherence to ARV treatment by HIV positive pregnant mother before and after delivery
2. To identify factors associated with poor ARV adherence in HIV positive pregnant mother
3. To assess the proportion of HIV exposed infants linked to services
4. To identify factors associated with linkage to HIV care services among HIV exposed infants

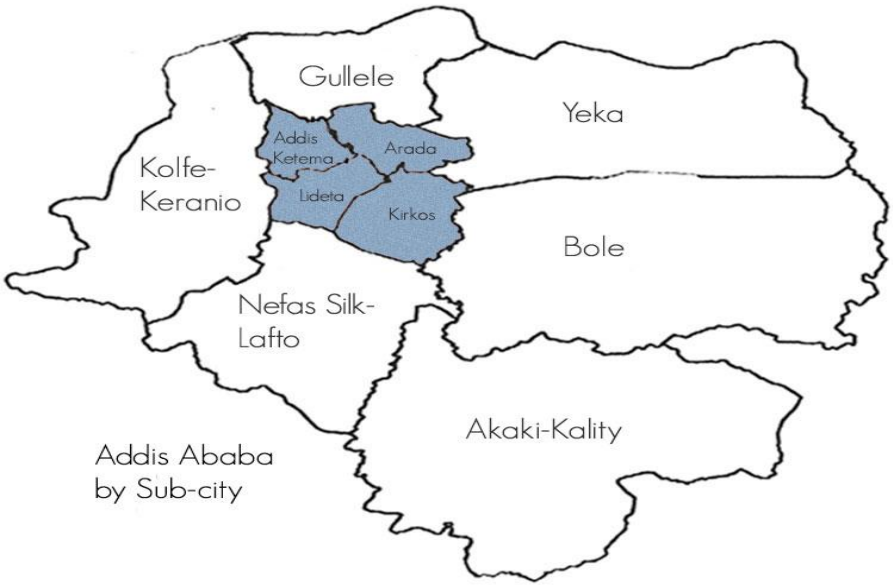
Peri- and post-partum PMTCT practices were not influenced by the study and following Ethiopian Guidelines. The study describes PMTCT procedures and evaluated outcome on an observational basis.

2. Methods

2.1 Study Sites

The study was conducted in Addis Ababa with a population of 3 million. The city is divided into 10 sub-cities. In 2013, 54 health facilities provided PMTCT service utilization in Addis Ababa, presently in 2015 73 health facilities provide PMTCT and 24 sites are under construction. The study took place in seven Health Center's (HC): Gulele HC, Kolfe HC, Sheromeda HC, Sarris HC, Bole HC, Mesholekya HC and Sarris HC. The seven health centers are located in sub cities bearing their names except for Sarris Heath Center HC is located in in Nifas Silk Lafto Subcity, Sheromeda is located in Gulele Subcity, and Mesholekya HC is located in Kirkos Subcity (Figure 1). The health center is a primary health care unit with in-patient and out-patient services including surgery, and laboratory services. A health center with PMTCT facilities can provide ARV prophylaxis, manage STI's, diagnose and treat UTI, anemia, TB , malaria and intestinal parasites, follow up on HIV exposed infants, attend to delivery, immunize infants, and counsel clients on HIV related matters. On average in each health center there are 4 nurses in the ANC department and 10 midwives in the delivery department and 3 nurses in Expanded Program on Immunization (EPI) department and 5 nurses working in the ART Department.

Figure 1. Addis Ababa is divided into 10 sub cities; Gulele, Yeka, Bole, Akaki- Kality, Nefas- Silk Lafto , Kolfe-Keranio, Addis Ketema, Lideta, Kirkos, Arada.



2.2 Study Design:

The Study is a prospective, observational cohort study of HIV positive mothers and their pregnancy through the time of delivery and until six weeks after delivery.

2.3 Study Populations:

HIV positive pregnant women that attended antenatal care service were recruited from seven health centers in Addis Ababa. The outcome analysis included recruited mothers and their infants.

2.4 Duration of the study

The recruitment period October 2012 and July 2015 and the total study period took approximately three years.

2.5 Eligibility criteria

2.5.1 Inclusion criteria

1. HIV positive pregnant mothers
2. Age 18 years or above
3. Willingness to provide information and data collected on their HIV status, PMTCT procedures, health status, personal characteristics as well as health information of their infant(s)
4. Voluntary written informed consent

2.5.2 Exclusion Criteria

1. Mental or physical incapacity (including ongoing labor) leading to inability to provide informed consent

2.6 Schedule of Events

The following flowchart summarizes study procedures including:

1. Recruitment of HIV positive pregnant woman at the antenatal care clinic

2. Perinatal assessments including pre- intra- and post-partum procedures

3. Early infant diagnostic procedures 6 weeks after delivery

All procedures are based on Ethiopian National PMTCT Guideline. Standard laboratory measurements will be based on local laboratory practice

Table 3: Schedule of Event

Procedures	**Ante-partum**	**Delivery**	**Post-partum**	
			Day-6	**Week-6**
Informed consent	x			
Tracing Information	x			
Baseline & Socio-demographic Information	x			
HIV History	x		x	x
CD4-count	x			
ARV Treatment History	x	x	x	x
Gestational Age, Onset of Labor, Premature Rupture of Membrane, Perinatal ARV	x	x		
Type, Duration and Complications of Delivery		x		
Infant Outcome (number of infants, APGAR, height, weight)		x		
Counseling Breastfeeding and Infant's ARV Prophylaxis		x	x	x
Adherence of ARV Infant Prophylaxis		x		
Adherence of Mother ARV Regimen			x	x
DBS-EID (infant)			x	x

2.7 Recruitment and inclusion procedures

Women presenting for antenatal care at participating health care facilities at the health centers were counseled and offered HIV antibody testing by a PMTCT nurse, as per the standard of care provided at that site. Women were provided introductory information about the study if either they were discovered to be HIV infected and agreed to receive their test results, or they already knew there HIV status from previous HIV testing. They enrolled as early as their first antenatal visit after their HIV infection is confirmed. At that point, the study was described to them as part of a screening and consent process. The following primary eligibility criteria were used to determine which women may be invited into the study:

- Age $\geq$ 18 years.
- Evidence of HIV infection, as documented by 2 positive EIA's; or 1 positive EIA; or 2 separate concurrent rapid tests. These are the WHO acceptable criteria for diagnosing HIV–1 infection in adults.

After the screening and consenting process, the study case report form's for Antepartum Visit -1 and a Compliance Questionnaire for PMTCT Antiretroviral Treatment Adherence was used to document the client's information. The Antepartum Case Report Form asked questions on demographics, HIV History, ARV history, obstetric and previous pregnancy, current pregnancy and lab diagnostics. The compliance questionnaire mainly concentrated on adherence to the ARV's on the last 4 days, last month.

2.8 Informed consent procedures

Patients underwent informed consent procedure prior to any study related activity. A consent form template in Amharic was provided to the client. The informed consent procedure was conducted by a study nurse. After the study was fully explained and answers were responded, written informed consent were obtained from the patient. In the case of illiterate study participant a thumb print was provided by the patient. The signed consent form was kept in in a locked cabinet throughout the study period. After the informed consent has been signed the first clinical Antepartum case report form was used to collect data from the client.

2.9 Adherence Questionnaires

Starting from the first visit antenatal care, delivery, at day 6 and week 6 the maternal ART status was asked and maternal adherence to antiretroviral treatment was assessed using a self-administered questionnaire. The 5-items questionnaire included (i) a 4-day recall asking for numbers of skipped doses (never, 1 , 2, 3 or 4 skipped doses), (ii) a 1-month recall of voluntary treatment interruptions (never, less than one weeks, 1 to 2 weeks, more than 2 weeks), (iii) an ordinal question of skipped doses and (iv) shifted doses more than two hours over the last month (never, rarely, often), and (v) a question about following prescription over the past month (totally followed, generally followed, often modified, almost never followed prescription, or interrupted treatment). Infant's assessment included up-take of Nevirapine (NVP) prophylaxis and adherence, using an adapted 5-items questionnaire related to difficulties in administering infant prophylaxis administered at day 6 and week 6 post-partum.

2.10 Data and sample collection during delivery

As a standard practice mothers delivered in the health centers where they received antenatal care. However, if the mother is transferred to another health facility or delivered at home, the data was collected when she came for her 6th day and 6th week postpartum visit. In the event that the mother cannot be found, tracing information was used by mother support group to re-establish contact. Intra-partum case report form and Adherence questionnaire was used to collect information on delivery and ARV taken during delivery. The Intrapartum Case Report form collected information on the type of delivery, newborn health, and ARV history.

2.11 Data and sample collection during the Postpartum 6 days and 6 weeks visit

According to the Guideline for Prevention of Mother to Child Transmission of HIV in Ethiopia 2011 post-partum care is required at 6 hours, 6 days and 6 weeks (6-6-6). Mother's came in for the first infant penta-valent vaccine and to have blood collected from the infant for EID at 6 weeks. All PMTCT Health centers have trained nurses that can collect blood using filter paper from the heels of infants. At 6 weeks mother support group coordinator according to follow-up dates called and reminded study clients of the EID collection dates**.** Data collection forms for Postpartum 6 day, 6 week, Infant Linkage and Adherence Questionnaire were filled out on 6 day and 6 week accordingly on the respective dates. Postpartum 6 day and 6 week questionnaires

collected data post- partum on mother health, child health and ARV history. Infant Linkage Questionnaire main focus was DBS Collection dates, DBS result received date, DBS result and infant linkage to care.

2.12 Laboratory procedures

Laboratory tests were performed at the respective health centers according to the national standard of care for HIV-positive pregnant mothers. All health centers are equipped with hematology, chemistry and CD4 analyzer. The standard of care for lab exams for HIV-a positive mother is a urine analysis test; hematology, liver function test and CD4 count. The lab results the study used was only for CD4 counts.

2.13 Statistical and Sample Consideration

An estimate of the sample size was computed based on a population proportion of 72% for adherence rate [107, 108]. A sample size of 310 pregnant women is found to be sufficient to achieve a confidence interval of 95% with a marginal error of 5%. The following formula was used to calculate the desired sample size.

$$n = \frac{(Z_{\alpha/2})^2 * p(1-p)}{\varepsilon^2}$$

Where,

$n = required sample size$

$Z\% = the\ \alpha/2\ standard\ normal\ distribution\ score, usually\ 95\%\ value (1.96)\ is\ consdered\ as\ appropriate$

$p = estimated\ value\ for\ the\ particular\ indicator (0.5\ in\ our\ case)$

$\varepsilon = margin\ of\ error, 5\%\ for\ our\ purpose$

In order to compensate for LFUP, 15% compensation was considered which increased our sample size to 357. Since we assumed that some respondents might not respond to all the questions, hence 5% non-response compensation was considered. The total amount of participants needed for the study after considering LFUP and non-response compensation was 375.

2.14 Sampling

Based on the recommendation of the city health bureaus sample health centers were selected purposively on the basis of high client flow. The quarterly reported number of HIV positive pregnant women attending health centers were considered in identifying the following study sites: Gulele HC (51), Kolfe HC (96), Kirkos HC (59), Sarris HC (111), Bole HC (49) , Mesholekya HC (70) and Sheromeda HC (58).

2.15 Data collection, management and analysis

Data was collected on case report forms (CRF), transcribed from questionnaires, lab reports, medical charts and interviews with patients. The questionnaires were pre-tested for reliability and validity by administering the questionnaire to a small number of people who resemble the population of interest. The information provided by the respondents was then be analyzed for the clarity, question wording, or response categories. Data collection was accomplished by 21 trained PMTCT nurses in seven health centers, that worked in either antenatal or delivery section. The study staff was trained for 1 full day on the purpose of the study, confidentiality, ethical consideration and data collection prior to the study start date

Data collection and analysis was done in close collaboration with key stakeholders including the health center PMTCT staff, Addis Ababa Regional Health Bureau and Addis Continental, Institute of Public Health. Only de-identified data was used in this analysis.

Clinical and laboratory data were recorded in study specific case report forms and entered into a study tailored database which was programmed in Epi Info (CDC Atlanta, Version 3.5.3). Data was double checked for validity by the researcher on several occasions during the study period. The data entry software included applicable edit checks such as valid values, range checks, skip patterns and inconsistency checking.

2.16 Statistical methodology

Descriptive statistics were reported for maternal baseline characteristics, HIV and ART associated information, infant outcome, infant prophylactic treatment and EID information. Treatment adherence was graded as good if all adherence questioners indicated optimal compliances (no interruption, never shifted or skipped doses, totally followed prescription);

moderate if doses were once or rarely skipped or shifted, or prescription were generally followed; poor if doses were more than once or often skipped or shifted, or prescription were often modified or worse. Adherence grades were assessed for mothers and infant for each time point (delivery, day 6 and week 6). Combined post-delivery or overall adherence was summarized, and discrepant adherence grades by different time-points were combined based on the worst case for adjacent grades (poor + moderate = poor) or using the middle value (poor + good = medium). Comparison of treatment adherence before and after delivery was performed using the McNemar's exact test. Study site adjusted Poisson regression with robust variance estimates was used to analyze socio-demographic and HIV related risk factors associated with poor antiretroviral treatment adherence. For all statistical tests an alpha level of <0.05 was used to define significance. All statistical analyses were performed using Stata statistics software (version 14, StataCorp, College Station, TX, USA).

2.17 Ethical Considerations

2.17.1 Involvement of Ethics Committees

The protocol and the informed consent document to be used in this study was submitted to the Addis Continental Institute of Public Health and the Addis Ababa Regional Health Bureau ethics committee's written documentation of approval of both the protocol and the informed consent was provided before starting the study.

The site principal investigator ensured that the purpose of the study is explained to the patient and that written consent is obtained prior to participation in the study. The patient and the study nurse signed the consent prior to entry into the study. The investigator retained a copy of the signed consent forms.

The investigator promptly reported to the Ethics Committee/IRB of all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and did not make changes in the research without Ethics Committee/IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

2.17.2 Risks and benefits

This was an observational study following procedures recommended by the Guidelines for Prevention of Mother to Child Transmission of HIV in Ethiopia, issued by the Federal Ministry of Health in 2011. Therefore, the risks of participating in this study did not exceed the risks induced by the routine health services. Additional risk by the study was minimal and is as follows: the primary risks to study participants are potential social harm from stigma. All study sites have had ongoing ART for at least one year to date. Stigma and potential harm from society were real causes of concerns in prior years of HIV care but presently due to behavioral change educations on HIV this problem has been reduced. Breach of confidentiality is greatest during enrollment and the interviewing stages of the study, steps were taken to mitigate that risk. In addition to procedures that were put in place to assure participant confidentiality, participants were not identified in any reports on this study and no patient identifying information were extracted from clinical records. Any copies of case report forms and data forms bearing any identifiers were kept in locked secured areas.

2.18 Participant identification

For the study each participant will received a unique study number which was the only identifier on the case report forms and within the database. A study Screening Log was maintained in which hospital identifier were linked to the study participant ID. The Screening Log was only accessible by assigned study staff and kept within the study regulatory binders.

2.19 Confidentiality and Privacy

Data abstraction forms and laboratory specimens were identified by coded number only, in order to maintain subject confidentiality. All records were kept locked. All computer entry and networking programs were done with coded numbers only.

2.20 Insurance

As a minimal risk study participant insurance was not requested.

2.21 Data Dissemination

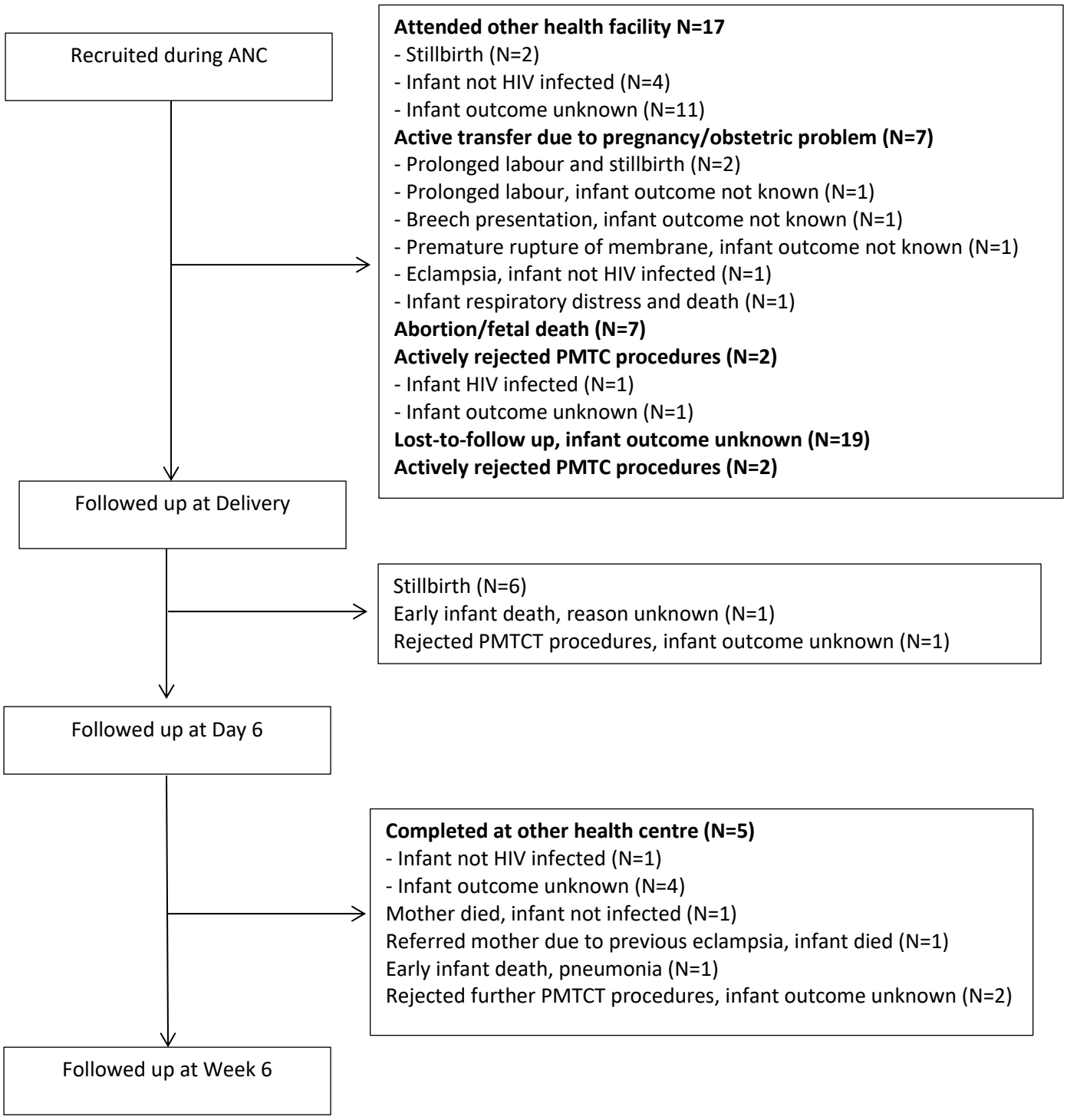
The study data is for a PhD thesis leading into a written report submitted to the Center of International Health at the University of Munich (LMU). After completion of the study, the data will be considered for reporting at local health forum, national and international scientific conferences or for publication in a scientific journal. The study PI will be responsible for these activities and will collaborate with the investigators and collaborators of the study to determine how the manuscript is written and edited, the number and order of authors, the journal to which it will be submitted and other related issues. Proposals for scientific presentations and publications need to be communicated with the study PI who will organize involvement and harmonization with the scientific study collaborators.

3. Results

3.1 Study Flow

Between October 2012 and July 2015 494 HIV infected pregnant women were enrolled into the study. Following study enrolment 17 (3.4%) women decided to continue PMTCT and obstetric services outside the study sites, 7 (1.4%) were actively transferred to higher level health facilities due to pregnancy of obstetric problems with reasons and infant outcome, and 7 (1.4%) did not continue with study procedures due to abortion or fetal death before the date of delivery (Figure 1). In addition, 2 women actively decided to not continue with PMTCT procedures and 19 women were lost to follow-up for unknown reasons before delivery. Of the remaining 442 women who were followed up during delivery 19 mother/infant pairs did no continue study procedures until week 6 post-partum either due continuation of post-natal services at other health facilities (N=5), still birth (N=6) or early infant death (N=3), maternal death (N=1), or rejection to continue with post-natal PMTCT procedures (N=3). Defining missed retention to PMTCT services for those women who at any time during the study period actively rejected PMTCT procedures (N=5) or were lost to follow-up without receiving tracing information (N=19), in total 24 (4.9%) women met these criteria throughout the entire study period. Reason for early termination of PMTCT services could not be determined for all of those women, frequent reasons which could be assessed were finical constrains, HIV stigma, distance of health center from home location, and rejection of ART.

Figure 2: Study Flow



3.2 Sociodemographic characteristics

Woman's study site affiliation, baseline demographic, socioeconomic, educational, occupational and HIV status characteristics are shown in Table 4. In brief, the median age was 28 years, the majority of women were married, Orthodox Christians, had primary or secondary school degrees, were house wives or private employees, with a median monthly family income of 1000 Ethiopian Birr (around 47 USD).

Table 4: Study site affiliations and baseline demographic, socioeconomic and HIV status characteristics of HIV infected, pregnant women enrolled into the study.

Variable		Total population N=494
Study sites	Sarris	111 (22.5)
	Kolfe	96 (19.4)
	Meshualekiya	70 (14.2)
	Kirkos	59 (11.9)
	Sheromeda	58 (11.7)
	Gulele	51 (10.3)
	Bole	49 (9.2)
Age (years)	Median (IQR)	28 (25-31)
	18-24 years	110 (22.3)
	25-29 years	202 (40.9)
	≥30 years	182 (36.8)
Marital Status	Single	55 (11.1)
	Married, Committed, Co-habitating	414 (83.8)
	Divorced, separated, widowed	25 (5.1)
Religion	Orthodox Christian	408 (82.6)
	Muslim	48 (9.7)
	Protestant or Catholic Christian	38 (7.7)
Ethnic tribe	Oromo	136 (27.5)
	Amhara	240 (48.6)
	Gurage	57 (11.5)
	Tigre	37 (7.5)
	Wolyata	11 (2.2)
	Other	13 (2.6)
Highest Educational level	No school degree	86 (17.4)
	Primary	96 (19.4)
	(post) Secondary	295 (59.7)
	No information	17 (3.5)

Values are provided for numbers (%), or medians (IQR) for continuous variables

Variable		Total population N=494
Current Occupation	House wife	239 (48.4)
	Private employee	108 (21.9)
	Daily labourer	49 (9.9)
	Government employee	35 (7.1)
	House maid	36 (7.3)
	Commercial sex worker	7 (1.4)
	Other	20 (4.1)
Monthly family income (Ethiopian Birr*)	median (IQR)	1000 (500-2000)
	0-500	127 (25.7)
	501-1500	190 (38.5)
	1501-2500	93 (18.8)

Values are provided for numbers (%), or medians (IQR) for continuous variables; * 1 US Dollar= 21.55 Ethiopian Birr (1000 Ethiopian Birr =46.41 USD)

3.3 HIV related characteristics and Gestational age during enrolment

Approximately half of the participants were in their third trimester when they were recruited in to the study. HIV infection was known before pregnancy among 263 (53.2%) women and HIV was diagnosed in 223 (45.1%) women during the current pregnancy Table 5.

A large proportion of the pregnant mothers initiated ART during current pregnancy, and only 3.1% initiated ART at or after delivery (Figure 2). Significant proportions of the mothers (91.1%) were either in WHO Stage I or II. The median CD4 count was 387 cells/µL and 14.8 % had a last CD4 count <200 cells/µL Table 5.

Table 5: Gestational age during enrolment and HIV related characteristics of pregnant women enrolled into the study in selected Addis Ababa Health Centers, Ethiopia, 2015

Variable		Total population N=494
Trimester entered into the study	1st trimester	44 (8.9)
	2nd trimester	200 (40.5)
	3rd trimester	250 (50.6)
Time of first HIV diagnosis	Before current pregnancy	263 (53.2)
	During current pregnancy	223 (45.1)
	Not known	8 (1.6)
Start of antiretroviral therapy	Before current pregnancy	166 (33.6)
	During/after current pregnancy	321 (65.0)
	Not known	7 (1.4)
ART regimen	TDF, 3TC, EFV	295 (60.6)
	ZDV, 3TC, NVP	83 (17.0)
	ZDV-mono therapy	37 (7.6)
	TDF, 3TC, NVP	35 (7.2)
	ZDV, 3TC, EFV	29 (6.0)
WHO-Stage	Stage 1/2	450 (91.1)
	Stage 3/4	44 (8.9)
Last CD4 count (cells/µL)	Median (IQR)	387 (261-536)
	<200 cells/µL	73 (14.8)
	200-<350 cells/µL	135 (27.3)
	≥350 cells/µL	268 (54.3)
	Missing data	18 (3.6)

Values are provided for numbers (%)

3.4 Maternal ART/PMTCT initiation and adherence

ART was initiated during or after the pregnancy in 321 of women, of those 94 (29.3%) were known HIV positive before the current pregnancy but still ART naïve, 220 (68.5%) women were HIV diagnosed during this pregnancy and for 7 women the data of HIV diagnosis was not known. In 166 women ART was already initiated before the current pregnancy. Most women received fixed dose combination of tenofovir, lamivudine and efavirenz, and 37 women still received zidovudine monotherapy following previous Option A procedures prior to the national transition into Option B+ policies (Table 5). The median time of ART initiation before delivery in women starting treatment during pregnancy was 17.4 weeks (IQR 11.7-23.9). Antiretroviral PMTCT coverage before delivery was critical in 19 (5.9%) women who had either no ART at delivery

(N=10) or very recently ART initiation (N=9) less than four weeks before delivery. In addition, 56 (17.4%) women initiated ART between 4 to 12 weeks before delivery accounting for suboptimal antiretroviral PMTCT coverage (Figure 4). Very late or late ART initiation in these 75 women was due to either late HIV diagnosis in 46 (61.3%), but due to delayed ART initiation despite known HIV diagnosis in 29 (38.7%) cases. The median time between HIV diagnosis and start of ART in women who were HIV diagnosed during pregnancy was 1.3 weeks (IQR 0-4.3), with 25.9% undergoing treatment initiation at the same day of HIV diagnosis, cumulatively 46.4 % started within one week and 74.1% within one month after diagnosis (Figure 5). Very late HIV diagnosis with less than 4 weeks before delivery or at the time of delivery was done in 6 (2.7%) and 3 (1.4%) women, respectively (Figure 3).

Figure 3: **Weeks of HIV diagnosis before delivery in HIV Infected, pregnant women who received first HIV diagnosis during pregnancy**

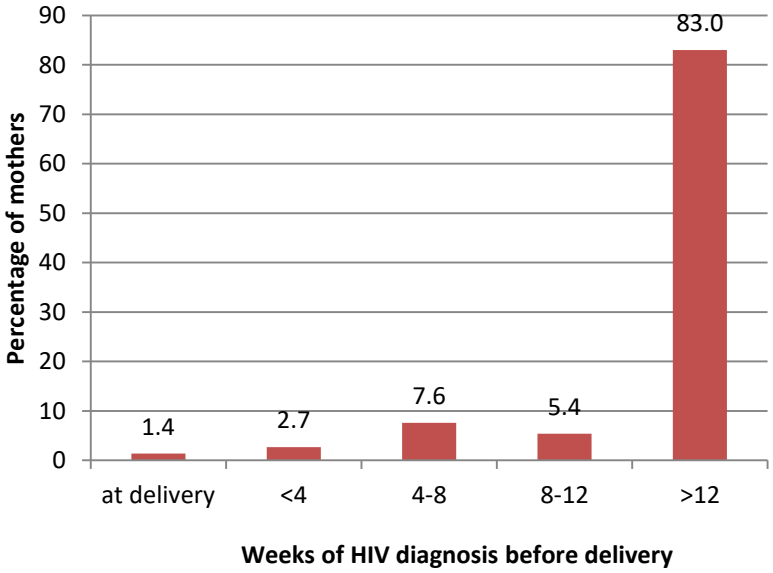


Figure 4: Weeks of ART initiation before delivery in HIV infected, pregnant women who initiated ART during pregnancy

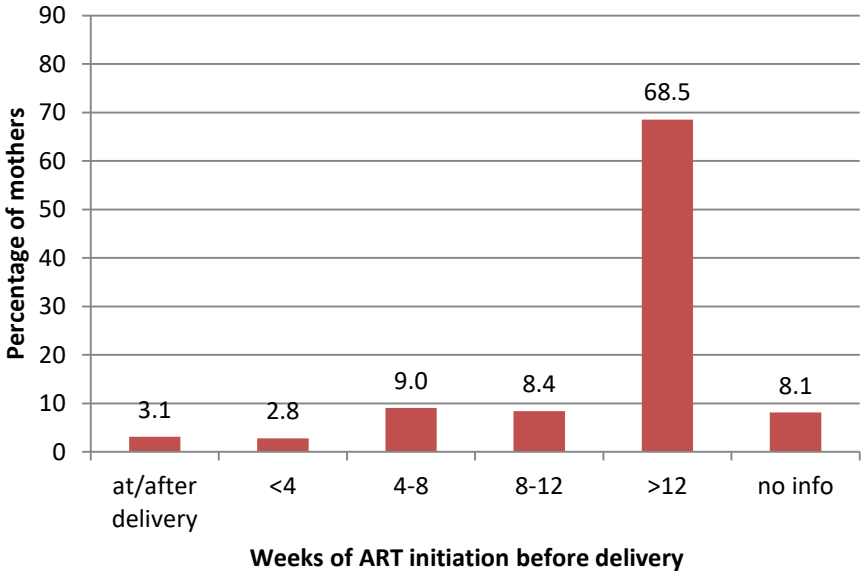
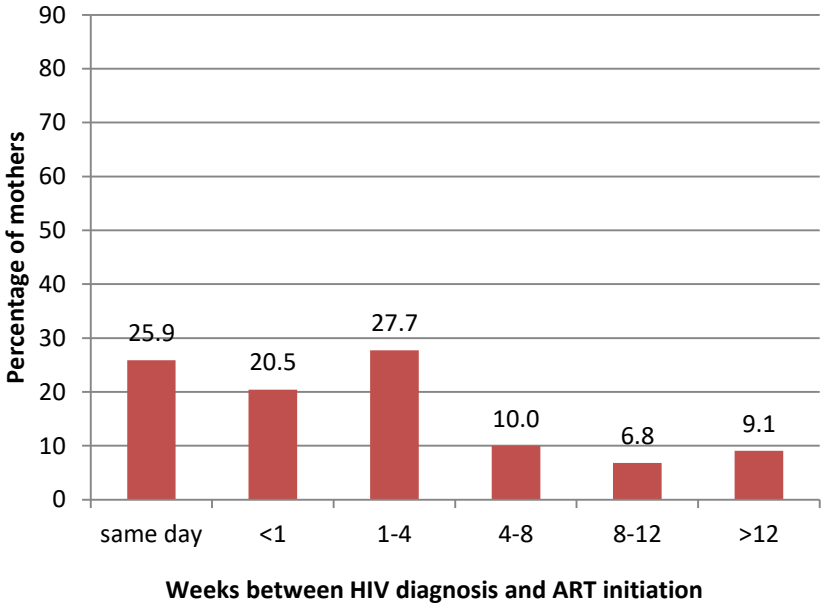


Figure 5: Weeks between HIV diagnosis and ART initiation in HIV infected, pregnant women who received first HIV diagnosis during pregnancy and initiated ART during pregnancy



3.5 Adherence and Risk factors associated with overall poor antiretroviral treatment adherence adjusted by study sites

Overall self-reported antiretroviral treatment adherence was graded as good in 41.3% and poor in 17.3% of women, and adherence was comparable between women who had started ART during pregnancy or were on ART already before pregnancy (Table 6). In contrast, treatment adherence was significantly better before delivery as compared to post-delivery (good adherence: 60.8% versus 50.5%, p= p<0.001; poor adherence: 7.0% versus 12.5%, p=0.002).

Table 6: Treatment adherence (overall, before and after delivery) in HIV infected pregnant women for the total population and stratified by ART initiation before or during the current pregnancy.

Variable	Total population N=494	ART initiation during/after pregnancy N=321*	ART initiation before pregnancy N=166*
ART adherence until delivery			
– good	296 (60.8)	200 (62.3)	96 (57.8)
– moderate	92 (18.9)	57 (17.8)	35 (21.1)
– poor	34 (7.0)	24 (7.5)	10 (6.0)
– missing information	65 (13.3)	40 (12.5)	25 (15.1)
ART adherence after delivery until Week- 6			
– good	246 (50.5)	168 (52.3)	78 (47.0)
– moderate	116 (23.8)	73 (22.7)	43 (25.9)
– poor	61 (12.5)	41 (12.8)	20 (12.0)
– missing information	64 (13.1)	39 (12.2)	25 (15.1)
Overall ART adherence			
– good	201 (41.3)	138 (43.0)	63 (38.0)
– moderate	138 (28.3)	88 (27.4)	50 (30.1)
– poor	84 (17.3)	56 (17.5)	28 (16.9)
– missing information	64 (13.1)	39 (12.2)	25 (15.1)

Values are provided for numbers (%); * N=7 women missing information on ART initiation

Study site adjusted risk factors associated with overall poor self-reported ART adherence are shown in Table 7. Socio-demographic factors significantly associated with poor treatment adherence included women who were divorced, separated or widowed (RR 2.2, 95% CI 1.3-3.8, p=0.004) and women who had access to low family income (RR 2.1, 95% CI 1.1-4.1, p=0.027), whereas age, religion, ethnicity, education, occupation, HIV diagnosis or ART initiation before or during pregnancy were not associated with poor adherence. In women starting ART during pregnancy poor treatment adherence was moderately associated with low CD4 counts <200

cells/µL (RR 1.7, 95% CI 1.0-3.0, p=0.49) and significantly associated with ART initiation during delivery (RR 2.5, 95% CI 1.1-5.6, p=0.028). No association was seen for WHO stages or if ART was immediately initiated within one week after HIV diagnosis.

Table 7: Demographic, socioeconomic, HIV status and antiretroviral risk factors associated with overall poor antiretroviral treatment adherence adjusted by study sites

		Poor ART adherence adjusted by study site		
Variable		**n/ N (%)**	**PR (95% CI)**	**p-value**
Age groups	18-24 years	24/93 (25.8)	1	-
	25-29 years	32 /174 (18.4)	0.7 (0.4-1.1)	0.156
	≥30 years	27/156 (17.3)	0.7 (0.4-1.1)	0.112
Marital status	Married, committed, cohabitating	69/355 (19.4)	1	-
	Single	5/47 (10.6)	0.5 (0.2-1.3)	0.167
	Divorced, separated, widowed	9/21 (42.9)	2.2 (1.3-3.8)	**0.004**
Religion	Orthodox Christian	73/274 (21.0)	1	-
	Muslim	3/40 (7.5)	0.4 (0.1-1.1)	0.066
	Protestant or catholic Christian	7/36 (19.4)	0.9 (0.5-1.9)	0.822
Ethnicity	Oromo	28/119 (23.5)	1	-
	Amhara	42/201 (20.9)	0.9 (0.6-1.4)	0.639
	Gurage	7/52 (13.5)	0.6 (0.3-1.2)	0.150
	Tigre	4/32 (12.5)	0.5 (0.2-1.4)	0.205
	Wolyata	0/9 (0)	-	-
	Other	2/8 (20)	0.8 (0.2-3.1)	0.804
Education	(post) Secondary	52/256 (20.3)	1	-
	Primary	16/82 (19.5)	1.0 (0.6-1.6)	0.880
	No school degree	11/68 (16.2)	0.8 (0.4-1.4)	0.449
Occupation	House Wife	44/212 (20.8)	1	-
	Private Employee	12/90 (14.4)	0.7 (0.4-1.2)	0.212
	Daily Labourer	6/40 (15.0)	0.7 (0.3-1.6)	0.409
	Government Employee	8/30 (26.7)	1.3 (0.7-2.5)	0.434
	House maid	4/29 (13.8)	0.7 (0.3-1.7)	0.399
	Commercial Sex Worker	2/7 (28.6)	1.4 (0.4-4.6)	0.609
	Other	6/15 (40.0)	1.9 (1.0-3.8)	0.064
Family income	>2500	10/76 (13.2)	1	-
	1501-2500	19/80 (23.8)	1.8 (0.9-3.6)	0.098
	501-1500	25/162 (15.4)	1.2 (0.6-2.3)	0.638
	0-500	29/105 (27.6)	2.1 (1.1-4.1)	**0.027**
HIV diagnosis	During pregnancy	37/195 (19.0)	1	-
	Before pregnancy	44/224 (19.6)	1.0 (0.7-1.5)	0.863
ART initiation	During pregnancy	55/281 (19.6)	1	-
	Before pregnancy	28/141 (19.9)	1.0 (0.7-1.5)	0.934

Table 8: Demographic, socioeconomic, HIV status and antiretroviral risk factors associated with overall poor antiretroviral treatment adherence adjusted by study sites for only women starting ART during pregnancy

		Poor ART adherence adjusted by study site		
Variable		**n/N (%)**	**PR (95% CI)**	**p-value**
WHO Stage	Stage 1/2	53/269 (19.7)	1	-
	Stage 3/4	2/12 (16.7)	0.9 (0.2-3.2)	0.848
CD4 count	≥350 cells/µL	28/153 (18.3)	1	-
	200-<350 cells/µL	10/77 (13.0)	0.7 (0.4-1.4)	0.313
	<200 cell/µL	14/44 (31.8)	1.7 (1.0-3.0)	0.049
ART start before delivery	>12 weeks	35 /209 (16.8)	1	-
	8-12 weeks	7/27 (25.9)	1.6 (0.8-3.2)	0.205
	4-8 weeks	6/27 (22.2)	1.3 (0.6-2.8)	0.501
	<4 weeks	3/8 (37.5)	2.2 (0.9-5.6)	0.097
	at/after delivery	4/10 (40.0)	2.5 (1.1-5.6)	0.028
ART initiation after HIV diagnosis	≥1 weeks after HIV diagnosis	38/188 (20.2)	1	
	<1 week after HIV diagnosis	15/89 (16.9)	0.8 (8.5-1.4)	0.512

3.6 Infant outcome and PMTCT procedures

Infant outcome information, was either directly obtained during study visits or through active tracing, 435 (88.1%) infants were born alive, 10 (2.0%) were stillbirths at delivery, there were 7 (1.4%) abortions or early intra-uterine fetal deaths, and for 42 (8.5%) infants no outcome information was obtainable. For infants born alive birth characteristics (gender, type of delivery, birth weight, 5-minutes Apgar score) are listed in Table 9. Four infants died during the post-natal period either due to respiratory distress, pneumonia or unknown reasons, for none of these infants HIV infection information was collected. Nevirapine prophylaxis was initiated in 98.6% of infants born alive, in 2 cases prophylaxis was not initiated due to early infant death or mother's refusal, and in 4 infants previously referred to other health facilities no information was obtainable. Self-reported adherence to NVP prophylaxis was good in the majority of patients, however, adherence reported at Week 6 was better than adherence reported at Day 6 (good adherence: 80.9% versus 76.6%, p=0.028; poor adherence: 3% versus 7.4%, p=0.003).

Table 9: Infant outcome, Nevirapine (NVP) prophylaxis and adherence in infants born alive. Data includes tracing information for infants who continued PMTCT procedures outside the study sites.

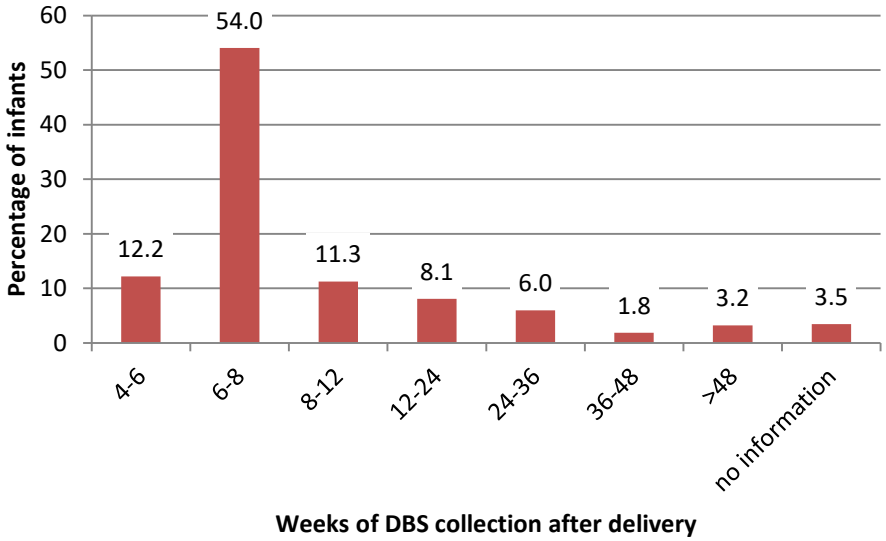
Variable		Life-born infants (N=435)
Gender	Female	212 (8.7)
	Male	218 (50.1)
	No information	5 (1.2)
Type of delivery	Spontaneous vaginal delivery	388 (89.2)
	Assisted vaginal delivery (vacuum/forceps)	1 (0.2)
	Caesarean section	41 (9.4)
	No information	5 (1.2)
Birth weight (g)	median (IQR)	3000 (2800-3500)
Apgar score 5 minutes	Normal (7-10)	410 (98.2)
	Low (4-6)	1 (0.2)
	No information	7 (1.6)
NVP prophylaxis	Started	429 (98.6)
	Not started	2 (0.5)
	Missing information	4 (0.9)
NVP adherence until Day 6 post-partum	Good	333 (76.6)
	Moderate	57 (13.1)
	Poor	32 (7.4)
	No information	13 (3.0)
NVP adherence between Day 6 and Week 6 post-partum	Good	352 (80.9)
	Moderate	53 (12.2)
	Poor	13 (3.0)
	No information	17 (3.9)
Infant Outcome	Not HIV infected	428 (98.4)
	HIV infected	3 (0.7)
	Early infant death	4 (0.9)

Values are provided for numbers (%), or medians (IQR) for continuous variables

3.7 Turnaround time for PCR analysis of Dry Blood Spots

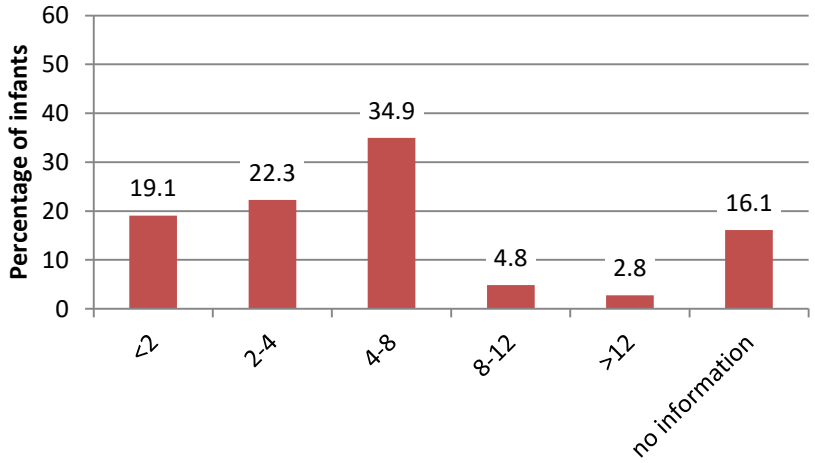
Dry blood spot (DBS) collection for early infants HIV diagnostics (EID) was performed after a medium duration of 6.7 weeks (range 3.4 to 74 weeks) following birth (Figure 6). DBS collection by week categories after delivery (Figure 6) indicating that only 12.2% had blood collected within the recommended period of 4 to 6 weeks post-partum, cumulative 68.2% until week 8, and 77.5% until week 12 post-partum. Eighty-three (19.1%) infants had DBS collected later than 12 weeks postpartum, 5% more than one year after delivery (Figure 6).

Figure 6: Proportion of infants born alive who received Dry Blood Spots Collection (DBS) at health facilities for Early Infant Diagnosis (EID) by weeks after delivery



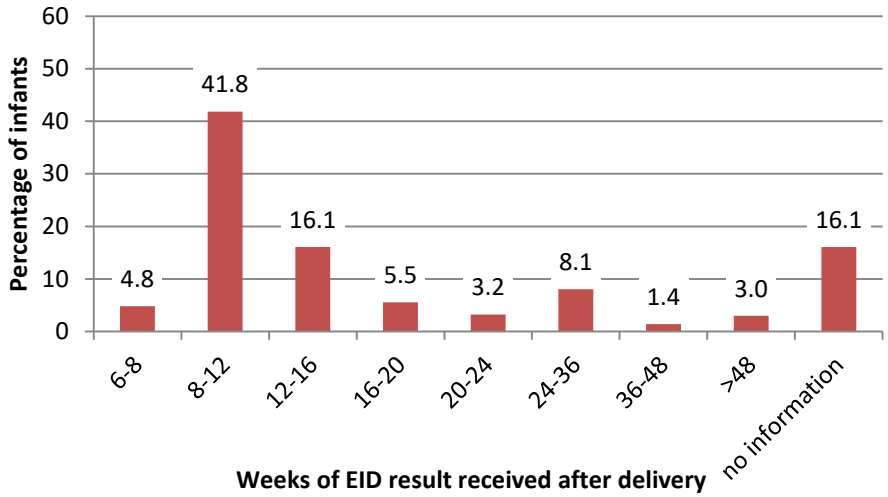
The turnaround time between DBS collection and receipt of EID results at the health center was in median 4.1 weeks (range 0.4 to 30.9 weeks) Figure 7. Turnaround times by week categories (Figure 7) indicating that cumulatively for 41.4% of infant's health centers had received EID results by week 4 and for 76.3% by week 8 after DBS collection. For 33 (7.6%) infants results were obtained later than 8 weeks after DBS collection (Figure 7).

Figure 7: Proportion of infants born alive who received EID results at health facilities by weeks after DBS Collection



The turnaround time between birth and receipt of EID was in median 11.3 weeks (range 6.7 to 78 weeks), week categories are shown in Figure 8. HIV results were available for cumulative 46.7% of infants within the first 12 weeks, and for 71.5% of infants within the first 24 weeks of life (Figure 8).

Figure 8: Proportion of infants born alive and who received EID results at health facilities by weeks after delivery



For 54 (12.5%) infants results were available after 24 weeks, for 4.4% even after one year Figure8. Out of 424 infants with available EID results only 2 (0.5%) were HIV-DNA reactive. In both cases mothers were on triple ART, infants received NVP prophylaxis, had DBS sample collection around week 7 and EID results were available at 9 and 10 weeks postpartum resulting into initiation of antiretroviral treatment. The third HIV infected infant was born from a mother, who terminated PMTCT during pregnancy, had home delivery and later DBS-EID procedures performed elsewhere with results available following tracing information. Therefore the overall transmission rate of 435 infants born alive was 0.7%, however, for 10 infants no HIV results were available either due to early infant death or missing outcome information.

4. Discussion:

In our prospective study of HIV infected, pregnant women and their new-born infants conducted at seven obstetric and PMTCT service delivering health facilities in Addis Ababa we observed a low HIV mother-to-child HIV transmission rate of 0.7% after a median 6.7 weeks indicating a high effectiveness of the current PMTCT procedures.

Previous studies from Ethiopian studies have reported transmission rates between 8.2 % and 9.5% in 2009 [102], the same author reported retrospectively assessed transmission rates of 14.3% by 2006, 14.9% by 2007 and 8.2% by 2009 [101]. Further retrospective analysis from the Gondar University Referral Hospital indicated 10% transmission between 2005 and 2011 [104] and from the Dil Chora Referral Hospital 15.7% between 2005 and 2013 [105].

Our data therefore reflects the beneficial impact of the latest recommendations on maternal and infant antiretroviral prevention in Ethiopia, namely associated with the introduction of maternal triple ART regimens. However, our study was conducted during the transition period from WHO 2010 to the 2013 Option B+ PMTCT procedures, and 7.6% of women still received the Option A regimen.

First data from the PROMISE study conducted in India, Malawi, South Africa, Tanzania, Uganda, Zambia and Zimbabwe reported similar transmission rates among women receiving triple drug regimens of 0.5% and 0.6% as compared to 1.8% among those receiving the Option A regimen [109].

The uptake of PMTCT procedures was very good in our study and only 2 women actively rejected procedures during pregnancy, in one this resulted into infant HIV transmission, and 3 after birth. Adding 19 further cases of lost to follow-up despite repeated tracing attempts, we concluded our study with a 4.9% loss of follow-up which is less then reported in other studies [111].

The majority of women in our study were HIV diagnosed and started on ART during pregnancy with a median duration of 1.3 weeks between HIV diagnosis and ART initiation, 46.4% of women starting treatment within one week after diagnosis. The immediate initiation or ART at the time of HIV diagnosis (test and treat) as recommended within the Option B+ procedures is

controversially debated as there is concern on sustained treatment adherence. In fact loss of treatment retention was reported to be higher in women starting on Option B+ as compared to those who were already on ART based on clinical and immunological criteria [111,112].

In our analysis we did not observe differences in self-reported treatment adherence in women who started ART within one week of HIV diagnosis as compared to those with later ART initiation, or in women who started ART during pregnancy as compared to those who were already on ART prior to pregnancy. As very late (less than 4 weeks prior to delivery) or late (less than 12 weeks prior to delivery) treatment initiation was noted in 75 women and due to delayed ART initiation despite known HIV infection in 38.7% of these cases , test and treat procedures might have avoided this deficiency.

The threshold duration of antenatal ART required for maximal PMTCT benefit is however unclear. The European Collaborative Study showed that 93.4% of women receiving NVP containing ART achieved viral suppression by 15 weeks [113]. A study of South African women initiating ART during pregnancy demonstrated that each additional week of treatment reduced the odds of perinatal HIV transmission by 8% [114], and the French Perinatal Cohort showed a reduction by 6% [115]. A retrospective cohort analysis of pregnant HIV-infected women in Zambia indicated that the optimal time of ART initiation should be in minimum 4 weeks but preferably 13 weeks prior to delivery [116].

The assessment of self-reported antiretroviral treatment adherence is often considered to be less reliable mainly leading to underestimation of adherence deficiency [123].

Nevertheless, in our study a substantial proportion of women admitted poor or moderate treatment adherence (overall 28.3% and 17.3%, respectively) which apparently did not lead to high mother-to-child transmission rates, however, may raise concern on treatment durability and development of drug resistance as reported in pregnant women from South Africa [117] and Malawi [118].

Evidence for decreased post-partum treatment adherence was also seen in our analysis, as the proportion of women with self-reported poor adherence was significantly higher prior to delivery as compared to post-partum treatment adherence. In addition, poor self-reported

adherence was significantly associated with women who started ART very late during the perinatal period, indicating that enforced adherence counselling after delivery is especially needed in these women. Other risk factors associated with poor treatment adherence were low CD4 counts, women who were separated, divorced or widowed, and women with very limited financial resources. Severe economic constraints represents most probably the most important risk factor in our population, which is affiliated with lack of transportation money to the health care facilities or lack of time to be spend for personal health care instead of generating income. In our study financial compensation for study visits were not provided as visits were based on the routine national antenatal and post-partum clinic appointments, however, financial support by health facilities was occasionally provided to those most in need. Interventions packages to increase the up-take and adherence to ART in Africa, including enforced patients counselling on HIV surrogate marker such as CD4 counts and strategies to meet economic challenges, are currently investigated in cluster-randomized trials such as the PopART Study (HPTN 071) [119].

Infant prophylactic NVP exposure in our study was very good and almost all infants started and complete treatments, possibly attributing to the low mother-to-child transmission rate observed. Self-reported adherence to NVP prophylaxis was better after one week as compared to the first six days following birth, possibly reflecting initial challenges to administer the NVP syrup appropriately.

In contrast the analysis of EID procedures in our study revealed major challenges. Although the time of infant dry blood sample collection (median 6.7 weeks after birth) and turnaround time EID results after DBS collection (median 4.1 weeks) was relatively reasonable, however not meeting the targeted 4-6 weeks for sample collection and 2 weeks turnaround time for EID results by guidelines, only 4.8% of EID results were available within the recommended two-months period following birth, and cumulatively 46.6% of EID results were available at the health facilities within three months. In a substantial proportion of cases EID results were received at much later time-points (cumulatively 12.5% later than 24 weeks after birth). The fact that that all infants alive received DBS collections and had reported EID results is most probably biased by study related perseverance. One of the major reasons for deficiencies in EID procedures was a stock out of reagents for EID testing at the centralized laboratory in Addis Ababa in 2013 which lasted for several months. Similar challenges are known form other

African countries as reflected within the UNAIDS report [1] and recently reviewed [120,121]. Challenges related to centralized EID procedures and linkage might be overcome with the introduction of novel point-of-care HIV diagnostic systems, which enable EID procedures directly at the health facilities and turnaround times of about one hour [122].

4.1 Conclusion

In conclusion our study demonstrated low mother-to-child HIV transmission rates in mothers who, for the majority, received triple ART during pregnancy associated with high coverage of infant NVP prophylactic treatment. Limitations for the generalizability of our results are mainly study procedure related, including selection bias of women who attended ANC and leaving out possibly most at risk cases who do not reach offered services, tracing procedures which might have affected our good PMTCT retention rates, repeated treatment counselling beyond the usual routine procedures, and perseverance to receive EID results. Despite that antiretroviral PMTCT coverage led to low transmission rates our data supports concerns of suboptimal treatment adherence possibly associated with later virological failure and drug resistance development which especially needs to be addressed during the post-partum period. Centralized EID procedures are complicated by linkage and infrastructural challenges which could be overcome with the implementation of novel EID point-of-care systems.

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6. Annex

6.1 Curriculum vitae (CV)

Name: Marshet Girma

DOB: September 11, 1968

Profession: Public Health Specialist

EDUCATION

6/02 - 5/03 **Johns Hopkins University School of Public Health, Baltimore, MD**

- Awarded Masters of Public Health Degree from the Department of International Health,
  May 2003
- Concentration in international health, infectious disease, epidemiology and biostatistics
- Masters Papers: "*A Quantitative Analysis of VCT Uptake in Addis Ababa Ethiopia in 2001"* and " *The Phenomena behind the Recurrence of Famine in Ethiopia"*

9/95 - 5/98 **California State University, Long Beach**

- Awarded Masters of Science in Biology , May 1998
- Awarded Certificate of Biotechnology in 1998
- Course emphasis in molecular Biology, biotechnology, medical microbiology
- Thesis Paper: "*Regulation of Phospholipid Synthesis in Cryptococcus neoformans"*

9/86 - 5/91 **Marymount Universities, Arlington, Virginia**

- Awarded Bachelors of Science in Biology , May 1991
- Course emphasis in molecular biology, biology, microbiology

List of Publication

1. Girma Marshet, Houng H-S, Edelman R, Bailey S, Barvir D, Bisbing J, Vaughn D, W Sun. 2001. Delayed Plaque Delayed Plaque Amplification and Taqman RT-PCR for Detecting Vaccine Viremia in Human Volunteers Given Live-Attenuated Tetravalent Dengue Vaccine. American Society of Tropical Medicine and Hygiene, Atlanta, Georgia, USA

2. Girma, Marshet and Cole, B. Timothy. 1998. *In vitro* and *in vivo* Studies of Phospholipid Synthesis in *Cryptococcus neoformans*. American Society for Microbiology 98th General Meeting, Atlanta, Georgia, U.S.A.

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4. Girma, Marshet and Klig, Lisa S. 1995. Effects of Metabolites Inositol and Choline on *Cryptococcus neoformans*. American Society for Microbiology Southern California Branch Meeting, San Diego, CA.

5. Udhayakumar V, Kariuki S, Kolczack M, Girma M, Roberts JM, Oloo AJ, Nahlen BL, Lal AA. Longitudinal study of natural immune responses to the Plasmodium falciparum apical membrane antigen (AMA-1) in a holoendemic region of malaria in western Kenya: Asembo Bay Cohort Project VIII. Am J Trop Med Hyg. 2001 Aug;65(2):100-7.

6.2 Statement on Pre-release and Contribution

The PhD *thesis "Effectiveness of prevention of mother-to child transmission (PMTCT) procedures in pregnant HIV infected women and their exposed infants at seven health centres in Addis Ababa "*, Ethiopia has not been previously been previously published or submitted for publication.

I, Marshet Girma have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND

I have drafted the work or revised it critically for important intellectual content; AND

I agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Lastly I want to thank my parents General Girma Felleke and Senait Gizaw who have worked very hard to be able to give all their children the best opportunities in life. I also want to thank my brother Feleke Girma for his generosity and kindness and helping me settle back in Ethiopia after 30 years of being away.

6.4 Affidavit

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I hereby declare, that the submitted thesis entitled :**Effectiveness of prevention of mother-to child transmission (PMTCT) procedures in pregnant HIV infected women and their exposed infants at seven health centres in Addis Ababa, Ethiopia**

is the result of my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

The submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

I further declare that the electronic version of the submitted thesis is congruent with the printed version both in content and format.

April 29, 2015

Place, Date

Signature of PhD Candidate